

## WHAT IS CLAIMED IS:

1. A method of functional brain mapping of a subject comprising the steps of:

- (a) illuminating an exposed cortex of a brain or portion thereof of the subject with incident light;
- (b) acquiring a reflectance spectrum of each picture element of at least a portion of the exposed cortex of the subject;
- (c) stimulating the brain of the subject;
- (d) during or after step (c) acquiring at least one additional reflectance spectrum of each picture element of at least the portion of the exposed cortex of the subject; and
- (e) generating an image highlighting differences among spectra of the exposed cortex acquired in steps (b) and (d), so as to highlight functional brain regions.

*Subj 11* 2. The method of claim 1, further comprising the step of using at least one filter to adjust the spectrum of the incident light.

3. The method of claim 1, wherein each of steps (b) and (d) is independently characterized by spectral resolution ranging between 1 nm and 50 nm and spatial resolution ranging between 0.1 mm and 1.0 mm.
4. The method of claim 1, wherein each of steps (b) and (d) is effected via an interferometer-based spectral imaging device.
5. The method of claim 1, wherein each of steps (b) and (d) is effected via a filters-based spectral imaging device.
6. *for a 2* The method of claim 1, further comprising the steps of generating individual spectra-images from spectra acquired in steps (b) and (d).
7. The method of claim 6, wherein said spectral-images are generated by attributing each of the pixels in the images a distinctive color or intensity according to oxygen saturation and/or blood volume characterizing its respective picture element in the cortex.

8. The method of claim 1, wherein the subject is awake.
9. The method of claim 1, wherein the subject is anesthetized.
10. The method of claim 1, wherein step (c) is effected by asking the subject to perform a task.
11. The method of claim 10, wherein said task is selected from the group consisting of reading, speaking, listening, viewing, memorizing, thinking and executing a voluntary action.
12. The method of claim 1, wherein step (c) is effected by a method selected from the group consisting of passively stimulating the brain through the peripheral nervous system of the subject and directly stimulating the cortex.
13. The method of claim 1, further comprising the step of generating an anatomical image of the exposed cortex and co-displaying

said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex.

14. The method of claim 13, wherein said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex are co-displayed side by side.
15. The method of claim 13, wherein said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex are superimposed.
16. The method of claim 1, wherein step (e) comprises a use of at least one threshold while generating the image highlighting differences among spectra of the exposed cortex acquired in steps (b) and (d).
17. The method of claim 1, wherein said image highlighting differences among spectra of the exposed cortex acquired in steps (b) and (d) is color or intensity coded.

18. The method of claim 1, wherein medical lines are connected to the subject on a single side thereof.

19. The method of claim 1, wherein medical lines are connected to the subject on a right or left side thereof.

20. The method of claim 1, wherein medical lines are connected to the subject at locations which are less communicating with the exposed portion of the cortex of the subject.

21. The method of claim 7, wherein said step (e) is characterized by highlighting oxygen saturation and/or blood volume differences of about at least 10 %.

22. The method of claim 7, wherein said step (e) is characterized by highlighting oxygen saturation differences and/or blood volume of about at least 5 %.

23. The method of claim 8, further comprising the step of also acquiring a reflectance spectrum of each picture element of at least the portion of the exposed cortex of the subject when the patient is briefly anesthetized.

24. The method of claim 1, wherein each of steps (b) and (d) is performed during at least  $N$  brain beats of the subject, wherein  $N$  is an integer selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten and an integer between and including eleven and forty.

25. The method of claim 1, wherein step (d) is executed more than about 3-5 seconds after initiation of step (c).

26. The method of claim 1, wherein step (d) is executed between about 5 and about 30 seconds after initiation of step (c).

27. The method of claim 1, wherein said stimulation prolongs about 5 to about 30 seconds.

28. The method of claim 1, wherein said stimulation prolongs about 10 to about 20 seconds.

29. The method of claim 5, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of intensity peaks or steeps characterizing one or more spectrally monitored substances.

30. The method of claim 5, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of intensity peaks or steeps characterizing hemoglobin selected from the group consisting of deoxy-hemoglobin, oxy-hemoglobin and deoxy-hemoglobin and oxy-hemoglobin.

31. The method of claim 30, wherein each of said filters is individually about 5 to about 15 nm full-width-at-half-maximum filter.

32. The method of claim 30, wherein each of said filters is individually about 10 nm full-width-at-half-max filter.

33. The method of claim 30, wherein said filters include N filters selected from the group consisting of an about 540 nm maximal transmittance filter, an about 575 nm maximal transmittance filter, an about 555 nm maximal transmittance filter, an about 513 nm maximal transmittance filter and an about 600 nm maximal transmittance filter, whereas N is an integer selected from the group consisting two, three, four and five.

34. The method of claim 33, wherein N equals two.

35. The method of claim 33, wherein N equals three.

36. The method of claim 33, wherein N equals four.

37. The method of claim 33, wherein N equals five.

38. The method of claim 30, wherein said filters include at least one multiple chroic filter.

39. The method of claim 30, wherein said filters include at least one filter of maximal transmittance at a wavelength which corresponds to at least one isosbestic point of deoxy-hemoglobin and oxy-hemoglobin and at least one additional filter of maximal transmittance at a wavelength which corresponds to at least one non-isosbestic point of deoxy-hemoglobin and oxy-hemoglobin.

40. The method of claim 1, wherein said reflectance spectrum of step (b) is an averaged reference spectrum of N measurements, wherein N is an integer and equals at least 2.

41. The method of claim 1, wherein said reflectance spectrum of step (d) is an averaged reference spectrum, wherein N is an integer and equals at least 2.

42. The method of claim 1, further comprising the steps of spatially registering spectral data acquired in steps (b) and (d).

43. The method of claim 1, wherein said image highlighting differences among spectra of the exposed cortex acquired in steps (b) and (d) is highlighting oxygen saturation and/or blood volume differences.

44. The method of claim 43, wherein step (e) comprises a use of at least one threshold while generating the image highlighting differences among spectra of the exposed cortex acquired in steps (b) and (d) of oxygen saturation and/or blood volume differences.

45. The method of claim 44, wherein said at least one threshold includes taking into account only picture elements in which, in step (b), in step (d) or both, an absolute oxygen saturation and/or blood volume is above a predetermined first threshold.

46. The method of claim 45, wherein said at least one threshold further includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined second threshold.

47. The method of claim 46, wherein clusters of neighboring picture elements above said first and said second threshold, said clusters include less than a predetermined number picture elements, are discarded.

48. The method of claim 44, wherein said at least one threshold includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined threshold.

49. The method of claim 44, wherein said at least one threshold is effected by discarding clusters of neighboring picture elements which include less than a predetermined number picture elements highlighting differences among spectra of the exposed cortex acquired in steps (b) and (d) of oxygen saturation and/or blood volume differences.

50. The method of claim 6, wherein said step of generating individual spectra-images from spectra acquired in steps (b) and (d) includes generating color or intensity coded saturation and/or blood volume maps.

51. The method of claim 50, wherein said coded saturation maps are coded oxygen saturation maps.

52. The method of claim 50, further comprising the step of generating an anatomical image of the exposed cortex and co-displaying at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex.

53. The method of claim 52, wherein said anatomical image is a monochromatic image.

54. The method of claim 52, wherein said anatomical image is a grayscale image.

55. The method of claim 52, wherein said anatomical image is a red-green-blue image.

56. The method of claim 52, wherein at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex are co-displayed side by side.

57. The method of claim 52, wherein at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex are superimposed.

58. The method of claim 1, wherein said image highlighting differences among spectra of the exposed cortex acquired in steps (b) and (d), so as to highlight functional brain regions, is coded via color or intensity so as to distinguish degree of said differences in accordance with at least one difference threshold.

59. The method of claim 13, wherein said anatomical image is a monochromatic image.

60. The method of claim 13, wherein said anatomical image is a grayscale image.

61. The method of claim 13, wherein said anatomical image is a red-green-blue image.

62. A method of generating an oxygen saturation and/or blood volume difference map of a tissue of a subject, the method comprising the steps of:

- (a) illuminating the tissue of the subject with incident light;
- (b) at a first time point, acquiring a spectrum of each picture element of the tissue of the subject;
- (c) at a second time point, acquiring at least one additional spectrum of each picture element of the tissue of the subject; and
- (d) generating an image highlighting differences among spectra of the tissue acquired in steps (b) and (c), so as to generate the oxygen saturation and/or blood volume difference map of the tissue.

63. The method of claim 62, wherein step (d) comprises a use of at least one threshold while generating the image highlighting differences among spectra of the tissue acquired in steps (b) and (c).

64. The method of claim 63, wherein said at least one threshold includes taking into account only picture elements in which, in step (b), in step (d) or both, an absolute oxygen saturation and/or blood is above a predetermined first threshold.

65. The method of claim 64, wherein said at least one threshold further includes taking into account only picture elements in which a difference in oxygen saturation and/or blood is above a predetermined second threshold.

66. The method of claim 65, wherein clusters of neighboring picture elements above said first and said second threshold, said clusters include less than a predetermined number picture elements, are discarded.

67. The method of claim 63, wherein said at least one threshold includes taking into account only picture elements in which a difference in oxygen saturation and/or blood is above a predetermined threshold.

68. The method of claim 62, wherein said at least one threshold is effected by discarding clusters of neighboring picture elements which include less than a predetermined number picture elements highlighting differences among spectra of the tissue acquired in steps (b) and (c).

69. The method of claim 62, further comprising the step of using at least one filter to adjust the spectrum of the incident light.

70. The method of claim 62, wherein each of steps (b) and (c) is independently characterized by spectral resolution ranging between 1 nm and 50 nm and spatial resolution ranging between 0.1 mm and 1.0 mm.

71. The method of claim 62, wherein each of steps (b) and (c) is effected via an interferometer-based spectral imaging device.

72. The method of claim 62, wherein each of steps (b) and (c) is effected via a filters-based spectral imaging device.

73. The method of claim 62, further comprising the steps of generating individual spectra-images from spectra acquired in steps (b) and (c).

74. The method of claim 73, wherein said spectral-images are generated by attributing each of the pixels in the images a distinctive color or intensity according to oxygen saturation and/or blood volume characterizing its respective picture element in the tissue.

75. The method of claim 62, wherein the tissue is selected from the group consisting of a brain, a heart, a liver, a kidney, an eye, a muscle and skin.

76. The method of claim 62, further comprising the step of oxygenating or deoxygenating the tissue between steps (b) and (c).

77. The method of claim 62, further comprising the step of generating an anatomical image of the tissue and co-displaying said oxygen saturation and/or blood volume difference map of the tissue with said anatomical image of the tissue.

78. The method of claim 77, wherein said oxygen saturation and/or blood volume difference map and the anatomical image of the tissue are co-displayed side by side.

79. The method of claim 77, wherein said oxygen saturation and/or blood volume difference map and said anatomical image of the tissue are superimposed.

80. The method of claim 62, wherein step (d) comprises a use of at least one threshold while generating said oxygen saturation and/or blood volume difference map.

81. The method of claim 62, wherein said oxygen saturation and/or blood volume difference map is color or intensity coded.

82. The method of claim 74, wherein said step (d) is characterized by highlighting oxygen saturation and/or blood volume differences of about at least 10 %.

83. The method of claim 74, wherein said step (d) is characterized by highlighting oxygen saturation and/or blood volume differences of about at least 5 %.

84. The method of claim 72, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of intensity peaks or steeps characterizing hemoglobin selected from the group consisting of deoxy-hemoglobin, oxy-hemoglobin and deoxy-hemoglobin and oxy-hemoglobin.

85. The method of claim 84, wherein each of said filters is individually about 5 to about 15 nm full-width-at-half-maximum filter.

86. The method of claim 84, wherein each of said filters is individually about 10 nm full-width-at-half-max filter.

87. The method of claim 84, wherein said filters include N filters selected from the group consisting of an about 540 nm maximal transmittance filter, an about 575 nm maximal transmittance filter, an about 555 nm maximal transmittance filter, an about 513 nm maximal transmittance filter and an about 600 nm maximal transmittance filter, whereas N is an integer selected from the group consisting two, three, four and five.

88. The method of claim 87, wherein N equals two.

89. The method of claim 87, wherein N equals three.

90. The method of claim 87, wherein N equals four.

91. The method of claim 87, wherein N equals five.

92. The method of claim 84, wherein said filters include at least one multiple chroic filter.

93. The method of claim 84, wherein said filters include at least one filter of maximal transmittance at a wavelength which corresponds to at least one isosbestic point of deoxy-hemoglobin and oxy-hemoglobin and at least one additional filter of maximal transmittance at a wavelength which corresponds to at least one non-isosbestic point of deoxy-hemoglobin and oxy-hemoglobin.

94. The method of claim 62, wherein said reflectance spectrum of step (b) is an averaged reference spectrum of  $N$  measurements, wherein  $N$  is an integer and equals at least 2.

95. The method of claim 62, wherein said reflectance spectrum of step (c) is an averaged reference spectrum, wherein  $N$  is an integer and equals at least 2.

96. The method of claim 62, further comprising the steps of spatially registering spectral data acquired in steps (b) and (c).

97. The method of claim 73, wherein said step of generating individual spectra-images from spectra acquired in steps (b) and (c) includes generating color or intensity coded oxygen saturation and/or blood volume maps.

98. The method of claim 97, further comprising the step of generating an anatomical image of the tissue and co-displaying at least one of said color or intensity coded oxygen saturation and/or blood volume maps and the anatomical image of the tissue.

99. The method of claim 98, wherein said anatomical image is a monochromatic image.

100. The method of claim 98, wherein said anatomical image is a grayscale image.

101. The method of claim 98, wherein said anatomical image is a red-green-blue image.

102. The method of claim 98, wherein at least one of said color or intensity coded oxygen saturation and/or blood volume maps and the anatomical image of the tissue are co-displayed side by side.

103. The method of claim 98, wherein at least one of said color or intensity coded oxygen saturation and/or blood volume maps and the anatomical image of the tissue are superimposed.

104. The method of claim 62, wherein said oxygen saturation and/or blood volume difference map is coded via color or intensity so as to distinguish degree of said differences in accordance with at least one difference threshold.

105. The method of claim 77, wherein said anatomical image is a monochromatic image.

106. The method of claim 77, wherein said anatomical image is a grayscale image.

107. The method of claim 77, wherein said anatomical image is a red-green-blue image.

108. A method of performing a neurosurgery for the removal of a mass from a brain of a subject while minimizing damage to a neighboring brain tissue, the method comprising the steps of:

- (a) performing a craniotomy so as to expose at least a portion of a cortex of the subject;
- (b) performing functional brain mapping of the subject by:
  - (i) illuminating the exposed portion of the cortex with incident light;
  - (ii) acquiring a reflectance spectrum of each picture element of at least a portion of the exposed cortex of the subject;
  - (iii) stimulating the neighboring brain tissue of the subject;
  - (iv) during or after step (iii) acquiring at least one additional reflectance spectrum of each picture

element of at least the portion of the exposed cortex of the subject; and

(v) generating an image highlighting differences among spectra of the exposed cortex acquired in steps (ii) and (iv), so as to highlight the functional brain regions of the neighboring brain tissue; and

(c) assisted by said image, removing the mass while minimizing damage to the neighboring brain tissue.

109. The method of claim 108, further comprising the step of using at least one filter to adjust the spectrum of the incident light.

110. The method of claim 108, wherein each of steps (ii) and (iv) is independently characterized by spectral resolution ranging between 1 nm and 50 nm and spatial resolution ranging between 0.1 mm and 1.0 mm.

111. The method of claim 108, wherein each of steps (ii) and (iv) is effected via an interferometer-based spectral imaging device.

112. The method of claim 108, wherein each of steps (ii) and (iv) is effected via a filters-based spectral imaging device.

113. The method of claim 108, further comprising the steps of generating individual spectra-images from spectra acquired in steps (ii) and (iv).

114. The method of claim 113, wherein said spectral-images are generated by attributing each of the pixels in the images a distinctive color or intensity according to oxygen saturation and/or blood volume characterizing its respective picture element in the cortex.

115. The method of claim 108, wherein the subject is awake.

116. The method of claim 108, wherein the subject is anesthetized.

117. The method of claim 108, wherein step (c) is effected by asking the subject to perform a task.

118. The method of claim 117, wherein said task is selected from the group consisting of reading, speaking, listening, viewing, memorizing, thinking and executing a voluntary action.

119. The method of claim 108, wherein step (c) is effected by a method selected from the group consisting of passively stimulating the brain through the peripheral nervous system of the subject and directly stimulating the cortex.

120. The method of claim 108, further comprising the step of generating an anatomical image of the exposed cortex and co-displaying said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex.

121. The method of claim 120, wherein said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex are co-displayed side by side.

122. The method of claim 120, wherein said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex are superimposed.

123. The method of claim 108, wherein step (e) comprises a use of at least one threshold while generating the image highlighting differences among spectra of the exposed cortex acquired in steps (ii) and (iv).

124. The method of claim 108, wherein said image highlighting differences among spectra of the exposed cortex acquired in steps (ii) and (iv) is color or intensity coded.

125. The method of claim 108, wherein medical lines are connected to the subject on a single side thereof.

126. The method of claim 108, wherein medical lines are connected to the subject on a single side thereof.

127. The method of claim 108, wherein medical lines are connected to the subject at locations which are less communicating with the exposed portion of the cortex of the subject.

128. The method of claim 114, wherein said step (v) is characterized by highlighting oxygen saturation and/or blood volume differences of about at least 10 %.

129. The method of claim 114, wherein said step (v) is characterized by highlighting oxygen saturation and/or blood volume differences of about at least 5 %.

130. The method of claim 115, further comprising the step of also acquiring a reflectance spectrum of each picture element of at least the portion of the exposed cortex of the subject when the patient is briefly anesthetized.

131. The method of claim 108, wherein each of steps (ii) and (iv) is performed during at least  $N$  brain beats of the subject, wherein  $N$  is an

integer selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten and an integer between and including eleven and forty.

132. The method of claim 108, wherein step (d) is executed more than about 3-5 seconds after initiation of step (c).

133. The method of claim 108, wherein step (d) is executed between about 5 and about 30 seconds after initiation of step (c).

134. The method of claim 108, wherein said stimulation prolongs about 5 to about 30 seconds.

135. The method of claim 108, wherein said stimulation prolongs about 10 to about 20 seconds.

136. The method of claim 112, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of

intensity peaks or steeps characterizing one or more spectrally monitored substances.

137. The method of claim 112, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of intensity peaks or steeps characterizing hemoglobin selected from the group consisting of deoxy-hemoglobin, oxy-hemoglobin and deoxy-hemoglobin and oxy-hemoglobin.

138. The method of claim 137, wherein each of said filters is individually about 5 to about 15 nm full-width-at-half-maximum filter.

139. The method of claim 137, wherein each of said filters is individually about 10 nm full-width-at-half-max filter.

140. The method of claim 137, wherein said filters include N filters selected from the group consisting of an about 540 nm maximal transmittance filter, an about 575 nm maximal transmittance filter, an about 555 nm maximal transmittance filter, an about 513 nm maximal

transmittance filter and an about 600 nm maximal transmittance filter, whereas N is an integer selected from the group consisting two, three, four and five.

141. The method of claim 140, wherein N equals two.

142. The method of claim 140, wherein N equals three.

143. The method of claim 140, wherein N equals four.

144. The method of claim 140, wherein N equals five.

145. The method of claim 137, wherein said filters include at least one multiple chroic filter.

146. The method of claim 137, wherein said filters include at least one filter of maximal transmittance at a wavelength which corresponds to at least one isosbastic point of deoxy-hemoglobin and oxy-hemoglobin and at least one additional filter of maximal transmittance

at a wavelength which corresponds to at least one non-isosbestic point of deoxy-hemoglobin and oxy-hemoglobin.

147. The method of claim 108, wherein said reflectance spectrum of step (b) is an averaged reference spectrum of  $N$  measurements, wherein  $N$  is an integer and equals at least 2.

148. The method of claim 108, wherein said reflectance spectrum of step (d) is an averaged reference spectrum, wherein  $N$  is an integer and equals at least 2.

149. The method of claim 108, further comprising the steps of spatially registering spectral data acquired in steps (ii) and (iv).

150. The method of claim 108, wherein said image highlighting differences among spectra of the exposed cortex acquired in steps (ii) and (iv) is highlighting oxygen saturation and/or blood volume differences.

151. The method of claim 150, wherein step (e) comprises a use of at least one threshold while generating the image highlighting differences among spectra of the exposed cortex acquired in steps (ii) and (iv) of oxygen saturation and/or blood volume differences.

152. The method of claim 151, wherein said at least one threshold includes taking into account only picture elements in which, in step (b), in step (d) or both an absolute oxygen saturation and/or blood volume is above a predetermined first threshold.

153. The method of claim 152, wherein said at least one threshold further includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined second threshold.

154. The method of claim 153, wherein clusters of neighboring picture elements above said first and said second threshold, said clusters include less than a predetermined number picture elements, are discarded.

155. The method of claim 151, wherein said at least one threshold includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined threshold.

156. The method of claim 151, wherein said at least one threshold is effected by discarding clusters of neighboring picture elements which include less than a predetermined number picture elements highlighting differences among spectra of the exposed cortex acquired in steps (ii) and (iv) of oxygen saturation and/or blood volume differences.

157. The method of claim 113, wherein said step of generating individual spectra-images from spectra acquired in steps (ii) and (iv) includes generating color or intensity coded saturation and/or blood volume maps.

158. The method of claim 157, wherein said coded saturation maps are coded oxygen saturation maps.

159. The method of claim 157, further comprising the step of generating an anatomical image of the exposed cortex and co-displaying at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex.

160. The method of claim 159, wherein said anatomical image is a monochromatic image.

161. The method of claim 159, wherein said anatomical image is a grayscale image.

162. The method of claim 159, wherein said anatomical image is a red-green-blue image.

163. The method of claim 159, wherein at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex are co-displayed side by side.

164. The method of claim 159, wherein at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex are superimposed.

165. The method of claim 108, wherein said image highlighting differences among spectra of the exposed cortex acquired in steps (ii) and (iv), so as to highlight functional brain regions, is coded via color or intensity so as to distinguish degree of said differences in accordance with at least one difference threshold.

166. The method of claim 120, wherein said anatomical image is a monochromatic image.

167. The method of claim 120, wherein said anatomical image is a grayscale image.

168. The method of claim 120, wherein said anatomical image is a red-green-blue image.

169. A system for functional brain mapping of a subject, the system comprising:

- (a) an illumination device for illuminating an exposed cortex of a brain or portion thereof of the subject with incident light;
- (b) a spectral imaging device for acquiring reflectance spectra of each picture element of at least a portion of the exposed cortex of the subject before and during and/or after stimulating the brain of the subject; and
- (c) an image generating device for generating an image highlighting differences among spectra of the exposed cortex acquired before and during and/or after stimulating the brain of the subject, so as to highlight functional brain regions.

170. The system of claim 169, further comprising at least one filter being engaged with said illumination device to adjust the spectrum of the incident light.

171. The system of claim 169, so designed and constructed so as to provide spectral resolution ranging between 1 nm and 50 nm and spatial resolution ranging between 0.1 mm and 1.0 mm.

172. The system of claim 169, wherein said spectral imaging device is an interferometer-based spectral imaging device.

173. The system of claim 169, wherein said spectral imaging device is a filters-based spectral imaging device.

174. The system of claim 169, wherein said image generating device is designed and constructed for generating individual spectral images from spectra of the exposed cortex acquired before and during and/or after stimulating the brain of the subject.

175. The system of claim 174, wherein said spectral-images are generated by attributing each of the pixels in the images a distinctive color or intensity according to oxygen saturation and/or blood volume and/or blood volume characterizing its respective picture element in the cortex.

176. The system of claim 169, wherein said image generating device is designed and constructed for generating an anatomical image of the exposed cortex and co-displaying said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex.

177. The system of claim 176, wherein said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex are co-displayed by said image generating device side by side.

178. The system of claim 176, wherein said image highlighting differences among spectra of the exposed cortex and the anatomical image of the exposed cortex are superimposed by said image generating device.

179. The system of claim 169, wherein said image generating device uses at least one threshold while generating the image highlighting differences among spectra of the exposed cortex.

180. The system of claim 169, wherein said image highlighting differences among spectra of the exposed cortex is color or intensity coded by said image generating device.

181. The system of claim 175, wherein said image generating device is set to highlight oxygen saturation and/or blood volume differences of about at least 10 %.

182. The system of claim 175, wherein said image generating device is set to highlight oxygen saturation and/or blood volume differences of about at least 5 %.

183. The system of claim 169, wherein said spectral imaging device is set for acquiring said reflectance spectra of each of said picture element of at least said portion of the exposed cortex of the subject before and during and/or after stimulating the brain of the subject during at least N brain beats of the subject, wherein N is an integer selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten and an integer between and including eleven and forty.

184. The system of claim 173, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of intensity peaks or steeps characterizing one or more spectrally monitored substances.

185. The system of claim 173, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of intensity peaks or steeps characterizing hemoglobin selected from the group consisting of deoxy-hemoglobin, oxy-hemoglobin and deoxy-hemoglobin and oxy-hemoglobin.

186. The system of claim 185, wherein each of said filters is individually about 5 to about 15 nm full-width-at-half-maximum filter.

187. The system of claim 185, wherein each of said filters is individually about 10 nm full-width-at-half-max filter.

188. The system of claim 185, wherein said filters include N filters selected from the group consisting of an about 540 nm maximal

transmittance filter, an about 575 nm maximal transmittance filter, an about 555 nm maximal transmittance filter, an about 513 nm maximal transmittance filter and an about 600 nm maximal transmittance filter, whereas N is an integer selected from the group consisting two, three, four and five.

189. The system of claim 188, wherein N equals two.

190. The system of claim 188, wherein N equals three.

191. The system of claim 188, wherein N equals four.

192. The system of claim 188, wherein N equals five.

193. The system of claim 185, wherein said filters include at least one multiple chroic filter.

194. The system of claim 185, wherein said filters include at least one filter of maximal transmittance at a wavelength which corresponds to

at least one isosbastic point of deoxy-hemoglobin and oxy-hemoglobin and at least one additional filter of maximal transmittance at a wavelength which corresponds to at least one non-isosbastic point of deoxy-hemoglobin and oxy-hemoglobin.

195. The system of claim 169, wherein said spectral imaging device is designed and constructed for spatially registering spectral data acquired thereby.

196. The system of claim 169, wherein said image generating device is designed and constructed for highlighting differences among oxygen saturation and/or blood volume of the cortex.

197. The system of claim 196, wherein said image generating device is designed for use of at least one threshold while generating the image highlighting differences among said oxygen saturation and/or blood volume of the cortex.

198. The system of claim 197, wherein said at least one threshold includes taking into account only picture elements in which, before, during and/or after said stimulation, an absolute oxygen saturation and/or blood volume is above a predetermined first threshold.

199. The system of claim 198, wherein said at least one threshold further includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined second threshold.

200. The system of claim 199, wherein clusters of neighboring picture elements above said first and said second threshold, said clusters include less than a predetermined number picture elements, are discarded.

201. The system of claim 197, wherein said at least one threshold includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined threshold.

202. The system of claim 197, wherein said at least one threshold is effected by discarding clusters of neighboring picture elements which include less than a predetermined number picture elements highlighting differences among oxygen saturation and/or blood volume of the cortex.

203. The system of claim 174, wherein said individual spectra-images are color or intensity coded saturation and/or blood volume maps.

204. The system of claim 203, wherein said coded saturation and/or blood volume maps are coded oxygen saturation and/or blood volume maps.

205. The system of claim 203, wherein said image generating device is designed and constructed for generating an anatomical image of the exposed cortex and co-displaying at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex.

206. The system of claim 205, wherein said anatomical image is a monochromatic image.

207. The system of claim 205, wherein said anatomical image is a grayscale image.

208. The system of claim 205, wherein said anatomical image is a red-green-blue image.

209. The system of claim 205, wherein at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex are co-displayed side by side.

210. The system of claim 205, wherein at least one of said color or intensity coded saturation and/or blood volume maps and the anatomical image of the exposed cortex are superimposed.

211. The system of claim 169, wherein said image generating device is designed and constructed to distinguish degree of said differences in accordance with at least one difference threshold.

212. The system of claim 176, wherein said anatomical image is a monochromatic image.

213. The system of claim 176, wherein said anatomical image is a grayscale image.

214. The system of claim 176, wherein said anatomical image is a red-green-blue image.

215. A system of generating an oxygen saturation and/or blood volume difference map of a tissue of a subject, the system comprising:

(a) an illumination device for illuminating the tissue of the subject with incident light;

- (b) a spectral imaging device for acquiring spectra of each picture element of the tissue of the subject at a first time point and at a second time point; and
- (c) an image generating device for generating an image highlighting differences among spectra of the tissue acquired in said first and said second time points, so as to generate the oxygen saturation and/or blood volume difference map of the tissue.

216. The system of claim 215, wherein said image generating device uses of at least one threshold while generating the image highlighting differences among spectra of the tissue acquired in said first and said second time points.

217. The system of claim 216, wherein said at least one threshold includes taking into account only picture elements in which, in said first time point, in said second time point, or both, an absolute oxygen saturation and/or blood volume is above a predetermined first threshold.

218. The system of claim 217, wherein said at least one threshold further includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined second threshold.

219. The system of claim 218, wherein clusters of neighboring picture elements above said first and said second threshold, said clusters include less than a predetermined number picture elements, are discarded.

220. The system of claim 216, wherein said at least one threshold includes taking into account only picture elements in which a difference in oxygen saturation and/or blood volume is above a predetermined threshold.

221. The system of claim 215, wherein said at least one threshold is effected by discarding clusters of neighboring picture elements which include less than a predetermined number picture elements highlighting differences among spectra of the tissue acquired in said first and said second time points.

222. The system of claim 215, further comprising at least one filter engaging said illumination device to adjust the spectrum of the incident light.

223. The system of claim 215, wherein spectral data acquired at each of said first and said second time points is independently characterized by spectral resolution ranging between 1 nm and 50 nm and spatial resolution ranging between 0.1 mm and 1.0 mm.

224. The system of claim 215, wherein spectral data acquired at each of said first and said second time points is collected via an interferometer-based spectral imaging device.

225. The system of claim 215, wherein spectral data acquired at each of said first and said second time points is collected via a filters-based spectral imaging device.

226. The system of claim 215, wherein said image generating device is designed and constructed for generating individual spectral-images from spectra acquired during said first and said second time points.

227. The system of claim 226, wherein said spectral-images are generated by attributing each of the pixels in the images a distinctive color or intensity according to oxygen saturation and/or blood volume characterizing its respective picture element in the cortex.

228. The system of claim 215, wherein said image generating device is designed and constructed for generating an anatomical image of the tissue and co-displaying said oxygen saturation and/or blood volume difference map of the tissue with said anatomical image of the tissue.

229. The system of claim 228, wherein said oxygen saturation and/or blood volume difference map and the anatomical image of the tissue are co-displayed side by side by said image generating device.

230. The system of claim 228, wherein said oxygen saturation and/or blood volume difference map and said anatomical image of the tissue are superimposed image generating device.

231. The system of claim 215, wherein said oxygen saturation and/or blood volume difference map is color or intensity coded.

232. The system of claim 225, wherein said filters-based spectral imaging device includes filters selected so as to collect spectral data of intensity peaks or steeps characterizing hemoglobin selected from the group consisting of deoxy-hemoglobin, oxy-hemoglobin and deoxy-hemoglobin and oxy-hemoglobin.

233. The system of claim 232, wherein each of said filters is individually about 5 to about 15 nm full-width-at-half-maximum filter.

234. The system of claim 232, wherein each of said filters is individually about 10 nm full-width-at-half-max filter.

235. The system of claim 232, wherein said filters include N filters selected from the group consisting of an about 540 nm maximal transmittance filter, an about 575 nm maximal transmittance filter, an about 555 nm maximal transmittance filter, an about 513 nm maximal transmittance filter and an about 600 nm maximal transmittance filter, whereas N is an integer selected from the group consisting two, three, four and five.

236. The system of claim 235, wherein N equals two.

237. The system of claim 235, wherein N equals three.

238. The system of claim 235, wherein N equals four.

239. The system of claim 235, wherein N equals five.

240. The system of claim 232, wherein said filters include at least one multiple chroic filter.

241. The system of claim 232, wherein said filters include at least one filter of maximal transmittance at a wavelength which corresponds to at least one isosbestic point of deoxy-hemoglobin and oxy-hemoglobin and at least one additional filter of maximal transmittance at a wavelength which corresponds to at least one non-isosbestic point of deoxy-hemoglobin and oxy-hemoglobin.

242. The system of claim 226, wherein said individual spectra-images are color or intensity coded oxygen saturation and/or blood volume maps.

243. A system for monitoring oxygen saturation in a tissue comprising a spectral imaging device and an image generating device, said spectral imaging device and said image generating device acting in synergy to produce an oxygen saturation difference map by highlighting tissue regions characterized by a characteristic selected from the group consisting of:

- (a) having an absolute or relative level of oxygen saturation above a predetermined first threshold;

- (b) having an oxygen saturation difference above a predetermined second threshold; and
- (c) having a cluster size above a predetermined size.

244. A system for monitoring oxygen saturation in a tissue comprising a spectral imaging device and an image generating device, said spectral imaging device and said image generating device acting in synergy to produce an oxygen saturation difference map by highlighting tissue regions characterized by:

- (a) having an absolute or relative level of oxygen saturation above a predetermined first threshold;
- (b) having an oxygen saturation difference above a predetermined second threshold; and
- (c) having a cluster size above a predetermined size.

245. A system for monitoring blood volume in a tissue comprising a spectral imaging device and an image generating device, said spectral imaging device and said image generating device acting in synergy to produce a blood volume difference map by highlighting tissue

regions characterized by a characteristic selected from the group consisting of:

- (a) having an absolute or relative level of blood volume above a predetermined first threshold;
- (b) having a blood volume difference above a predetermined second threshold; and
- (c) having a cluster size above a predetermined size.

246. A system for monitoring blood volume in a tissue comprising a spectral imaging device and an image generating device, said spectral imaging device and said image generating device acting in synergy to produce a blood volume difference map by highlighting tissue regions characterized by:

- (a) having an absolute or relative level of blood volume above a predetermined first threshold;
- (b) having a blood volume difference above a predetermined second threshold; and
- (c) having a cluster size above a predetermined size.

247. A system for functional brain mapping comprising a spectral imaging device and an image generating device, said spectral imaging device and said image generating device acting in synergy to produce an anatomical image of the brain or a portion thereof and a coded functional map of the brain or said portion thereof, said coded functional map reflecting a change in the brain in response to a stimulus, said functional map and said anatomical image being co-displayed.

248. The method of claim 62, wherein said reflectance spectrum of step (b) is an averaged reference spectrum of  $N$  brain beats, wherein  $N$  is an integer and equals at least 2.

249. The method of claim 1, wherein said reflectance spectrum of step (b) is an averaged reference spectrum of  $N$  brain beats, wherein  $N$  is an integer and equals at least 2.

250. The method of claim 108, wherein said reflectance spectrum of step (b) is an averaged reference spectrum of  $N$  brain beats, wherein  $N$  is an integer and equals at least 2.

251. A method of brain mapping of a subject comprising the steps of:

- (a) illuminating an exposed cortex of a brain or portion thereof of the subject with incident light;
- (b) acquiring a reflectance spectrum of each picture element of at least a portion of the exposed cortex of the subject; and
- (e) generating an image highlighting concentrations of at least one substance in the brain.

*a*

252. The system of claim 251, wherein a plurality of images highlighting differences among spectra are displayed either superimposed, overlaid or integrated.

253. The method of claim 1, wherein a plurality of images highlighting differences among spectra are displayed either superimposed, overlaid or integrated.

254. The method of claim 5, wherein step (a) is effected by an illumination device operated with an alternating current characterized by a frequency time.

255. The method of claim 254, wherein (i) an exposure time of all filters of said filters-based spectral imaging device is substantially equal; and (ii) an exposure time of each of said filters is a multiplicity of said frequency time by an integer.

256. The method of claim 62, wherein a plurality of images highlighting differences among spectra are displayed either superimposed, overlaid or integrated.

257. The method of claim 72, wherein step (a) is effected by an illumination device operated with an alternating current characterized by a frequency time.

258. The method of claim 257, wherein (i) an exposure time of all filters of said filters-based spectral imaging device is substantially equal;

and (ii) an exposure time of each of said filters is a multiplicity of said frequency time by an integer.

259. The method of claim 108, wherein a plurality of images highlighting differences among spectra are displayed either superimposed, overlaid or integrated.

260. The method of claim 112, wherein step (b)(i) is effected by an illumination device operated with an alternating current characterized by a frequency time.

261. The method of claim 260, wherein (i) an exposure time of all filters of said filters-based spectral imaging device is substantially equal; and (ii) an exposure time of each of said filters is a multiplicity of said frequency time by an integer.

262. The system of claim 169, wherein a plurality of images highlighting differences among spectra are displayed either superimposed, overlaid or integrated.

263. The system of claim 173, wherein said illumination device is operated with an alternating current characterized by a frequency time.

264. The system of claim 263, wherein (i) an exposure time of all filters of said filters-based spectral imaging device is substantially equal; and (ii) an exposure time of each of said filters is a multiplicity of said frequency time by an integer.

265. The system of claim 215, wherein a plurality of images highlighting differences among spectra are displayed either superimposed, overlaid or integrated.

266. The system of claim 225, wherein said illumination device operated with an alternating current characterized by a frequency time.

267. The system of claim 266, wherein (i) an exposure time of all filters of said filters-based spectral imaging device is substantially equal;

and (ii) an exposure time of each of said filters is a multiplicity of said frequency time by an integer.

268. The method of claim 13, wherein the anatomical image includes text identifying brain portions.

269. The method of claim 1, wherein at least one orientation element is placed on the exposed portion of the cortex prior to step (b), so as to provide orientation.

270. The method of claim 269, wherein said at least one orientation element also serves as a white target.

271. The method of claim 270, wherein at least a portion of said at least one orientation element has an index of refraction close to an index of refraction of the cortex.